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# Opportunities for exercise during pullet rearing, Part II: Long-term effects on bone characteristics of adult laying hens at the end-of-lay

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**ABSTRACT** Osteoporosis in laying hens has been a production and welfare concern for several decades. The objective of this study was to determine whether differing opportunities for exercise during pullet rearing influences long-term bone quality characteristics in end-of-lay hens. A secondary objective was to assess whether differing opportunities for exercise in adult housing systems alters bone quality characteristics in end-of-lay hens. Four flock replicates of 588 Lohmann Selected Leghorn-Lite pullets were reared in either conventional cages (Conv) or an aviary rearing system (Avi) and placed into conventional cages (CC), 30-bird furnished cages (FC-S), or 60-bird furnished cages (FC-L) for adult housing. Wing and leg bones were collected at the end-of-lay to quantify bone composition and strength using quantitative computed tomography and bone breaking strength (BBS). At the end-of-lay, Avi hens had greater total and cortical cross-sectional area ( $P < 0.05$ ) for the radius and tibia, greater total

bone mineral content of the radius ( $P < 0.001$ ), and greater tibial cortical bone mineral content ( $P = 0.029$ ) than the Conv hens; however, total bone mineral density of the radius ( $P < 0.001$ ) and cortical bone mineral density of the radius and tibia ( $P < 0.001$ ) were greater in the Conv hens. Hens in the FC-L had greater total bone mineral density for the radius and tibia ( $P < 0.05$ ) and greater trabecular bone mineral density for the radius ( $P = 0.027$ ), compared to hens in the FC-S and CC. Total bone mineral content of the tibia ( $P = 0.030$ ) and cortical bone mineral content of the radius ( $P = 0.030$ ) and tibia ( $P = 0.013$ ) were greater in the FC-L compared to the CC. The humerus of Conv hens had greater BBS than the Avi hens ( $P < 0.001$ ), and the tibiae of FC-L and FC-S hens had greater BBS than CC hens ( $P = 0.006$ ). Increased opportunities for exercise offered by the aviary rearing system provided improved bone quality characteristics lasting through to the end-of-lay.

**Key words:** laying hen, bone health, housing system, exercise, pullet rearing

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## INTRODUCTION

Osteoporosis in laying hens has been a prominent welfare concern since the 1980s because of the relationship between osteoporosis and high fracture incidence during the laying period (Thorp, 1994; Fleming et al., 1998b; Knowles and Wilkins, 1998; Sandilands et al., 2009), and during catching and handling at de-

population (Gregory et al., 1992). The onset of osteoporosis is thought to begin between 16 and 31 wk of age when histological evidence demonstrates that the amount of cancellous (trabecular) bone volume declines by approximately 50% as a shift towards the production of medullary bone takes over. Sustained egg production is believed to further increase the prevalence of osteoporosis between 31 to 42 wk of age as the skeletal calcium reserves are substantially eroded (Cransberg et al., 2001), coinciding with reports of an increase in fractures around 40 wk of age (Fleming, 2008).

The effects of osteoporosis are in most cases visible only when the case is severe, first described as “cage layer fatigue” (Couch, 1955), resulting in spontaneous immobility, mortalities, and poor eggshell quality. As the amount of structural mineralized bone tissues decreases during the laying period, the bones increase in fragility and are more susceptible to fractures

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(Whitehead, 2004). In laying hens, fractures have the potential to limit mobility and feed and water intake (Nasr et al., 2012a), increase pressure on the spinal cord (Riddell et al., 1968), restrict the normal respiratory process by having fresh or healed fractures of the ribs and keel bone (Duncker, 2000; Codd et al., 2005; Claessens, 2009), and are likely to cause acute and chronic pain (Nasr et al., 2012b). Mortalities related to osteoporosis are believed to be a result of both paralysis from spinal degeneration and the development of improper muscle function due to lack of metabolic calcium reserves (Whitehead, 2004).

In attempts to reduce the widespread occurrence of osteoporosis in laying hens, several research avenues have yielded positive results. Dietary changes to calcium-phosphorous ratios (Tyler, 1940a, 1940b; Summers et al., 1976; Rennie et al., 1997), supplemental large particle calcium strategies (Fleming et al., 1998a; Koutoulis et al., 2009; Saunders-Blades et al., 2009; Cufadar et al., 2011), and genetic selection for high bone quality lines (Bishop et al., 2000; Hocking et al., 2003; Fleming et al., 2006; Podisi et al., 2012) have all been moderately successful for reducing the severity of osteoporosis. Providing opportunities for load-bearing exercise for adult laying hens by the addition of perches (Tauson and Abrahamsson, 1994; Abrahamsson et al., 1996; Enneking et al., 2012; Hester et al., 2013) and elevated dust baths (Jendral et al., 2008) to cages, and housing hens in aviary systems with greater opportunities for locomotion and flight (Whitehead and Wilson, 1992; Newman and Leeson, 1998) improve bone strength and composition. As opportunities for loading increase, so does bone size; the opposite effect is also true — disuse or absence of loading leads to reduced bone size and thickness characteristics over time, as demonstrated by confined housing in conventional cage systems (Shipov et al., 2010).

Although providing a more complex adult housing system has offered measured success to improvements in bone health, an additional approach to preventing osteoporosis involves targeting the pullet stage, during the period of musculoskeletal development. In human medicine, osteoporosis is increasingly being considered a pediatric disease, with the precursors developing in childhood and adolescence and the outward signs of the disease manifesting in adulthood (Bailey et al., 1999). Epidemiological determinants of osteoporosis in women include the amount of peak bone mass in adolescence, the maintenance of peak bone mass in middle age, and the overall rate of bone loss (Chesnut, 1989). Increasingly, studies focusing on the effects of exercise on the amount of bone mass developed during childhood and adolescence in combination with exercise and bone mass levels in adulthood have become a key avenue of research in human medicine for the prevention and reduction of osteoporosis (Vincente-Rodriguez, 2006; Burrows, 2007).

Recently, the same concept has shifted the focus toward providing exercise during the rearing phase of pul-

lets to build a stronger skeleton with a greater capacity for structural bone growth and medullary bone calcium reserves, thereby reducing skeletal depletion later in life. Preliminary evidence has demonstrated that rearing in non-cage systems improves the overall bone composition and strength of pullet bones at 16 wk (Regmi et al., 2015; Casey-Trott et al., 2017b) and reduces overall keel bone damage throughout the laying phase (Vits et al., 2005; Casey-Trott et al., 2017a). The addition of perches during rearing in conventional cages also provided some long term benefits to bone health in 71-week-old hens (Hester et al., 2013), and Regmi et al. (2016) reported that a positive effect on bone composition detected at 16 wk was maintained during the laying phase.

The objective of this study was to assess the effect of differing opportunities for exercise during the pullet rearing phase on the long-term bone quality characteristics of laying hens. Pullets were reared in either an aviary that allowed for running, jumping, perching, wing-flapping, and flight starting at one d of age, or standard, conventional rearing cages offering limited opportunities for exercise throughout the rearing period. A secondary objective was to identify interactions between opportunities for exercise during rearing with those afforded in the adult housing system. Placement of pullets from the aviary and conventional rearing cages into 2 sizes of large, furnished cages or conventional layer cages allowed for a  $2 \times 3$  factorial arrangement with replication of rearing treatment in multiple cage units. This experimental design also allowed for comparison of the effect of increased exercise in the form of greater total cage area on bone quality. To the best of our knowledge, this is the first study to carry out a controlled, longitudinal assessment of the effect of exercise during rearing on the long-term bone quality characteristics of several consecutive flocks, of a single strain, raised from one d of age to the end-of-lay on a single site with identical feeding and lighting. Results on effects of rearing treatment on musculoskeletal characteristics from a sample of birds at 16 wk (Casey-Trott et al., 2017b) and prevalence of keel fractures throughout the laying period in the same population of hens used in the current study (Casey-Trott et al., 2017a) are reported elsewhere.

## MATERIALS AND METHODS

The effects of rearing system, standard cage (**Conv**) or rearing aviary (**Avi**), and adult housing system, conventional cage (**CC**), 30-bird furnished cage (**FC-S**), or 60-bird furnished cage (**FC-L**), were tested using a  $2 \times 3$  factorial arrangement with rearing flock replicated in 4 blocks over time. Each of the 4 rearing flocks contributed 3 replicate cages to each of the adult housing systems. Animal use was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #1947).

## ***Pullet Housing and Management***

Four consecutive flocks of Lohmann Selected Leghorn Lite (**LSL-Lite**) were obtained from a commercial hatchery at d one. For each consecutive flock, 960 pullets were conveniently selected for rearing in standard, conventional cages (Ford Dickinson, Mitchell, Ontario, Canada; 16 pullets/cage during wk zero to 6 with a space allowance of 145 cm<sup>2</sup>/pullet followed by 8 pullets/cage during wk 6 to 16 with a space allowance of 290 cm<sup>2</sup>/pullet; total cage area = 2,322 cm<sup>2</sup>), and half were conveniently selected for rearing in a Portal Pullet rearing system (Clark Ag Systems, Caledonia, Ontario, Canada; 756 pullets/aviary enclosure; system space allowance of 285 cm<sup>2</sup>/pullet during wk zero to 6; total system + outer platforms + litter space allowance of 754 cm<sup>2</sup>/pullet during wk 6 to 16). Details regarding the pullet housing systems and management protocols can be found in Casey-Trott et al. (2017b).

## ***Adult Laying hen Housing and Management***

At 16 wk of age, 294 pullets from each rearing system (Avi and Conv) from each flock (1 to 4) were weighed and transferred to 2 adult housing rooms, each holding 12 Farmer Automatic Enrichable (Furnished) Cages (Clark Ag Systems), and one adult housing room holding 90 standard, conventional cages, of which 12 standard, conventional cages (Ford Dickinson) were included in the study. In both the furnished cage rooms, and the conventional cage room, 2 flocks were housed simultaneously (Flocks 1 and 2; Flocks 3 and 4) with the placement into all cages equally balanced for all 4 flocks. In all rooms, a conveniently selected group of hens from a single rearing treatment (Avi or Conv) was placed into each cage, balancing both rearing treatments equally within each room. Each furnished cage room contained 6 large, furnished cages (60 hens, total area = 41,296 cm<sup>2</sup>, 688 cm<sup>2</sup>/hen) and 6 small, furnished cages (30 hens, total area = 20,880 cm<sup>2</sup>, 696 cm<sup>2</sup>/hen). Each bank of 6 cages had 3 tiers with one large and one small cage on each tier. The conventional room contained 12 standard conventional cages of equal size (8 hens/cage; total area = 4,025 cm<sup>2</sup>, 503 cm<sup>2</sup>/hen), all on a single tier. The same rearing and adult rooms were used for each consecutive flock.

All flocks were fed identical, standard commercial layer crumbled pellet diets (18% CP, 4.22% Ca, 0.44% available P) with automatic feed chains running every 3 h commencing at the start of a 14-hour light period from 05:00-19:00 h with 15-minute sunrise and sunset starting at 05:00 h and 18:45 h, respectively. In the furnished cage rooms, the light intensity varied among tiers, with the highest intensity recorded at the top tiers measuring 10 to 15 lux and the lowest intensity at the bottom tiers measuring 4 to 5 lux. Each rearing treatment was balanced for tier. Each furnished cage provided a curtained nest area proportional to cage size (94 cm<sup>2</sup>/hen), 10 cm high perches (15 cm<sup>2</sup>/hen) run-

ning parallel to the cage front throughout the middle area, and a smooth, plastic scratch area (large: 42 cm<sup>2</sup>/hen; small: 83 cm<sup>2</sup>/hen). Nipple drinkers with cups were located above the feed auger down the middle of the cage. The feed troughs (12 cm/hen) were located on both outer sides of the cages. Conventional cages were equipped with 2 nipple drinkers running down the middle of the cages, with the feed troughs (8 cm/hen) on the outer side of the cage. All rooms were sealed and entirely lit with artificial light (incandescent) with no natural, external light sources.

## ***Bone Sample Collection***

At 73 wk of age, the room lights were dimmed to 30% for ease of collection, and 10% of the hens from each furnished cage (FC-L: n = 6; FC-S: n = 3; Total n = 54/ flock) from all 4 flocks were conveniently selected from various regions within the cage, weighed, euthanized by cervical dislocation, and frozen for later bone collection. To prevent bone breakage due to wing flapping during convulsions, all birds were restrained using a handmade wrap comprised of cotton cloth and Velcro to contain wing flapping. All hens from each conventional cage (n = 8; Total N = 48/flock) were collected, weighed, euthanized by cervical dislocation, and frozen at -20°C for later bone collection.

To collect the bones, hens were thawed and the right and left radius, humerus, and tibia were removed. The bones from the right side were used for quantitative computed tomography (**QCT**) analysis and the bones from the left side were used to test bone breaking strength (**BBS**). Only one freeze and thaw cycle was allowed for all bones. Immediately after the bones were extracted, a subset of 8 conveniently selected bones per rearing by adult housing system treatment per flock (N = 48/flock: Conv/FC-L: n = 8; Conv/FC-S: n = 8; Conv/CC: n = 8; Avi/FC-L: n = 8; Avi/FC-S: n = 8; Avi/CC: n = 8), from the right side were placed into 10% formalin for >7 d for later QCT analysis. All the bones from the left side were placed in a moving air fume hood to air dry for >7 d for later analysis of BBS (Newman and Leeson, 1998). If the bone on the left side was fractured, the right bone was used instead. No bones with visible fractures were used for either the QCT or BBS analysis.

## ***Quantitative Computed Tomography***

A Stratec XCT<sup>3</sup> scanner (Model 922010; Norland Medical Systems Inc., Fort Atkinson, WI) with XMENU software version 5.40C was used for analysis of bone density (mg/cm<sup>3</sup>) and area (mm<sup>2</sup>) of the total bone, cortical bone, and trabecular bone. Measurements of the trabecular space presumably include both medullary and trabecular bone (Korver et al., 2004). Details regarding the QCT techniques can be found in Casey-Trott et al. (2017b).



### Three-point Bone Breaking Strength

An Instron Dynamic and Static Materials Test System (Model # 4204; Instron Corp., Canton, MA) with Automated Materials Test System software was used to assess BBS. Details regarding the BBS techniques can be found in Casey-Trott et al. (2017b).

### Statistical Analysis

All statistical analyses were completed using SAS statistical software version 9.4 (SAS Institute, Cary, NC). The level for assessment of statistical significance of differences between means was set at  $P < 0.05$ .

Rearing and adult BW at depopulation were analyzed separately to determine the relationship of rearing and adult housing system on BW for Flocks 1 to 4. To assess rearing BW, a general linear mixed model analysis (PROC MIXED command) was used with rearing system (Avi vs, Conv) as a fixed effect and Flock number (1, 2, 3, 4) as a random effect. For adult BW, PROC MIXED was used with rearing system (Avi, Conv), adult housing system (FC-L, FC-S, CC), and the interaction between the 2, included in the model as fixed effects and Flock number (1, 2, 3, 4) as a random effect. Rearing and adult BW were not included as covariates in the further analyses assessing adult laying hen bone characteristics, as rearing system had an effect on rearing BW ( $P = 0.042$ ) and adult BW ( $P = 0.049$ ), and, therefore, rearing system was not considered to be independent from rearing or adult BW.

Bone quality data were analyzed using a general linear mixed model analysis (PROC MIXED command). The QCT and BBS response variables were assessed with rearing system (Avi, Conv) and adult housing system (FC-L, FC-S, CC) and the interaction between the 2 as fixed effects. Flock number (1, 2, 3, 4) was included in the model as a random effect.

All data were tested for normality and normality of residuals using PROC UNIVARIATE command and no data required transformation. The trabecular density and trabecular bone mineral content of the humerus could not be assessed due to the presence of minimal to no trabecular or medullary bone present in the humerus, as it is primarily a pneumatic bone.

## RESULTS

There was an effect of rearing system on rearing BW, with the Avi pullets having a lower mean BW (1,213.2 g  $\pm$  14.8 SE) at placement into the adult housing systems than Conv pullets (1,240.7 g  $\pm$  14.7 SE;  $P = 0.042$ ); however, placement of pullets was balanced for BW with no difference between adult housing treatments (FC-L: 1,225.0 g  $\pm$  15.5 SE; FC-S: 1,243.9 g  $\pm$  19.5 SE; CC: 1,223.8 g  $\pm$  15.5 SE;  $P = 0.555$ ).

Rearing system also had an effect on the BW of the adult hens at 73 wk of age. Hens that were reared in the pullet aviary had a higher mean adult BW (1,838.1

g  $\pm$  27.3 SE) compared to hens that were reared in conventional cages (1,770.1 g  $\pm$  26.0 SE;  $P = 0.049$ ). Adult housing system did not have an effect on the adult BW (FC-L: 1,823.4 g  $\pm$  28.8 SE; FC-S: 1,807.8 g  $\pm$  29.4 SE; CC: 1,781.1 g  $\pm$  25.5 SE;  $P = 0.473$ ).

### Effect of Rearing System on Bone Characteristics

The total density of the radius at 73 wk of age was greater in the Conv hens compared to the Avi hens ( $P < 0.001$ ; Table 1). The cortical density at 73 wk of age of the radius ( $P < 0.001$ ; Table 1), humerus ( $P < 0.001$ ; Table 2), and tibia ( $P < 0.001$ ; Table 3) were greater in the Conv hens than the Avi hens.

Rearing system had the opposite effect on bone cross-sectional area. The total cross-sectional area at 73 wk of the radius ( $P < 0.001$ ; Table 1), humerus ( $P < 0.001$ ; Table 2), and tibia ( $P = 0.019$ ; Table 3), was less in the Conv hens compared to the Avi hens. The same pattern was found for the cortical cross-sectional area of the radius ( $P < 0.001$ ; Table 1) and tibia ( $P = 0.003$ ; Table 3), and the trabecular cross-sectional area of the radius ( $P < 0.001$ ; Table 1) and humerus ( $P < 0.001$ ; Table 2).

The radius of the Avi hens had a greater total bone mineral content ( $P < 0.001$ ; Table 1) and greater trabecular bone mineral content ( $P < 0.001$ ; Table 1) than the Conv hens at 73 wk of age. The tibia of the Avi hens had a greater cortical bone mineral content ( $P = 0.029$ ; Table 3), but a lower trabecular bone mineral content ( $P = 0.039$ ; Table 3) than the Conv hens at the end-of-lay. Rearing system did not affect any of the bone mineral content measures for the humerus of adult hens (Table 2).

There were no significant interactions between rearing and adult housing systems for any of the QCT measures (Tables 1–3).

### Effect of Adult Housing System on Bone Characteristics

Adult housing system had an effect on several QCT bone characteristics for the radius and tibia, but not the humerus. Total bone mineral density was greatest in the FC-L compared to both the FC-S and CC for both the radius ( $P = 0.013$ ; Table 1) and tibia ( $P < 0.001$ ; Table 3). Trabecular bone mineral density was also greatest in the FC-L with no difference between the FC-S and CC for the radius ( $P = 0.027$ ; Table 1). There was no effect of adult housing system on total or cortical cross-sectional area for the radius (Table 1), humerus (Table 2), or tibia (Table 3). The trabecular cross-sectional area of the tibia was least in the FC-L with no difference between the FC-S and CC ( $P = 0.003$ ; Table 3). The total bone mineral content was greater in the FC-L compared to the CC for the tibia ( $P = 0.030$ ; Table 3) with the FC-S having an intermediate

**Table 1.** Bone traits of the radius in 73-week-old laying hens subjected to different rearing and adult housing systems as measured by quantitative computed tomography.

		Density mg/cm <sup>3</sup> (±SE)			Cross-sectional area mm <sup>2</sup> (±SE)			Bone mineral content mg/mm (±SE)		
		Total	Cortical	Trabecular	Total	Cortical	Trabecular	Total	Cortical	Trabecular
Rearing <sup>1</sup>										
Conv		730.1 (16.74)	990.5 (7.85)	223.9 (7.72)	5.8 (0.12)	3.9 (0.14)	1.9 (0.12)	4.2 (0.15)	3.9 (0.14)	0.44 (0.03)
Avi		616.7 (17.50)	910.6 (8.35)	220.8 (8.11)	7.6 (0.12)	4.5 (0.15)	3.1 (0.13)	4.7 (0.15)	4.1 (0.15)	0.65 (0.03)
	DF	114	114	113	114	114	114	114	114	114
	F-value	40.14	64.30	0.12	181.90	17.30	45.63	16.52	2.76	23.89
	P-Value	<0.001	<0.001	0.727	<0.001	<0.001	<0.001	<0.001	0.099	<0.001
Adult housing <sup>2</sup>										
FC-L		708.5 (17.78) <sup>a</sup>	962.2 (8.54)	237.3 (8.27) <sup>a</sup>	6.6 (0.13)	4.4 (0.14)	2.2 (0.14)	4.6 (0.16)	4.2 (0.15) <sup>a</sup>	0.50 (0.03)
FC-S		653.6 (22.77) <sup>b</sup>	947.6 (11.62)	217.4 (10.91) <sup>b</sup>	6.8 (0.17)	4.1 (0.18)	2.7 (1.9)	4.3 (0.18)	3.9 (0.18) <sup>a,b</sup>	0.58 (0.05)
CC		658.2 (17.14) <sup>b</sup>	941.8 (8.07)	212.5 (7.94) <sup>b</sup>	6.7 (0.12)	4.1 (0.14)	2.6 (0.13)	4.3 (0.15)	3.9 (0.15) <sup>b</sup>	0.56 (0.03)
	DF	114	114	113	114	114	114	114	114	114
	F-value	4.50	2.02	3.73	0.33	2.35	3.01	3.05	3.61	1.14
	P-Value	0.013	0.137	0.027	0.717	0.100	0.053	0.051	0.030	0.323
Interaction R*A <sup>3</sup>										
Conv * FC-L		760.1 (23.39)	998.5 (12.03)	243.1 (11.25)	5.6 (0.17)	3.9 (0.19)	1.7 (0.20)	4.3 (0.19)	3.9 (±0.19)	0.38 (0.05)
Conv * FC-S		708.1 (27.07)	995.8 (14.18)	214.4 (13.12)	5.7 (0.20)	3.7 (0.21)	2.1 (0.24)	4.0 (0.21)	3.7 (±0.21)	0.45 (0.06)
Conv * CC		722.2 (21.18)	977.1 (10.63)	214.4 (10.21)	5.9 (0.15)	3.9 (0.17)	2.0 (0.17)	4.2 (0.18)	3.9 (±0.17)	0.49 (0.04)
Avi * FC-L		656.9 (21.31)	925.9 (10.75)	231.5 (10.15)	7.6 (0.16)	4.8 (0.17)	2.8 (0.18)	5.0 (0.18)	4.4 (±0.17)	0.62 (0.04)
Avi * FC-S		599.0 (32.76)	899.3 (17.50)	220.5 (16.01)	7.8 (0.25)	4.4 (0.25)	3.3 (0.30)	4.6 (0.25)	3.9 (±0.25)	0.71 (0.07)
Avi * CC		594.2 (21.17)	906.5 (10.63)	210.6 (10.06)	7.5 (0.15)	4.2 (0.17)	3.2 (0.17)	4.4 (0.18)	3.8 (±0.17)	0.63 (0.04)
	DF	114	114	113	114	114	114	114	114	114
	F-value	0.24	0.54	0.28	1.69	2.24	0.02	2.46	1.82	0.98
	P-Value	0.784	0.584	0.754	0.189	0.111	0.980	0.090	0.166	0.378

<sup>a,b</sup>Within a column, for each treatment variable (Adult housing, Interaction R\*A), means lacking a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Pullets housed in a conventional rearing system (Conv) or an aviary rearing system (Avi) until 16 wk of age, Flocks 1 to 4.

<sup>2</sup>Adult hens placed into furnished cage- large (FC-L), furnished cage-small (FC-S), or conventional cages (CC) from 16 to 73 wk of age, Flocks 1 to 4.

<sup>3</sup>Interaction between rearing housing system and adult housing system.

value. The cortical bone mineral content of the radius ( $P = 0.030$ ; Table 1) and tibia ( $P = 0.013$ ; Table 3) followed the same pattern with the greatest values reported in the FC-L compared to the CC, and FC-S with an intermediate value.

The results for the BBS of the humeri, radii, and tibiae are presented in Table 4. Rearing system had an effect only on the breaking strength of the humerus ( $P < 0.001$ ), with Conv hens exhibiting a greater BBS ( $9.2 \text{ kg} \pm 0.45 \text{ SE}$ ) compared to Avi hens ( $6.3 \text{ kg} \pm 0.44 \text{ SE}$ ) at 73 wk of age. Adult housing system had an effect only on the BBS of the tibia ( $P < 0.006$ ) with a higher breaking strength of hens in FC-L and FC-S ( $14.8 \text{ kg} \pm 0.76$  and  $14.4 \text{ kg} \pm 0.78 \text{ SE}$ ) than hens in CC ( $13.5 \text{ kg} \pm 0.77 \text{ SE}$ ) at 73 wk of age.

## DISCUSSION

Based on the results from the QCT analysis, aviary-reared hens maintained greater values for the majority of the bone composition measures through to the end-of-lay compared to conventionally reared hens. This study was part of a larger research project using the same population of LSL-Lite hens described in Casey-Trott et al. (2017b). Some of the improved bone parameters reported in the Avi pullets at 16 wk (Casey-Trott et al., 2017b) were maintained in adult laying hens at 73 wk, suggesting that providing regular opportunities for varied load-bearing exercise during

pullet rearing has substantial, lifelong effects on the bone composition of adult laying hens. Additionally, bone mineral deposition in the radius and tibia can be stimulated further by continued opportunities for exercise during the adult laying period for hens in FC-L.

## Effect of Rearing System on Bone Characteristics

The greater cross-sectional area and bone mineral content of the aviary-reared pullets at 16 wk of age (Casey-Trott et al., 2017b) carried over into adulthood; however, bone mineral density in adult hens was higher in the Conv hens. Similar results have been reported describing greater bone areas in hens housed in furnished cages (Jendral et al., 2008) and free-range systems (Shipov et al., 2010) compared to conventional cages with less bone mineral density. The greater adult bone mineral density in Conv hens in the present study is likely due to the same amount of bone mineral production, but in a bone with a smaller cross-sectional area compared to the larger cross-sectional area of the bones of the Avi hens. This result coincides with the overall wider wing and leg bones as determined by larger trabecular, cortical, and total bone cross-sectional area reported in the aviary-reared pullets compared to conventionally reared pullets (Casey-Trott et al., 2017b). The typically higher total and cortical bone mineral content values for the adult radii and

**Table 2.** Bone traits of the humerus in 73-week-old laying hens subjected to different rearing and adult housing systems as measured by quantitative computed tomography.

Density mg/cm <sup>3</sup> (±SE)				Cross-sectional area mm <sup>2</sup> (±SE)			Bone mineral content mg/mm (±SE)			
		Total	Cortical	Trabecular	Total	Cortical	Trabecular	Total	Cortical	Trabecular
<b>Rearing<sup>1</sup></b>										
<b>Conv</b>		115.0 (14.78)	989.0 (6.65)	–	55.2 (1.10)	11.5 (0.40)	42.6 (1.14)	6.3 (0.73)	11.4 (0.39)	–
<b>Avi</b>		95.9 (17.20)	933.6 (8.06)	–	65.9 (1.28)	12.3 (0.48)	51.6 (1.32)	5.9 (0.85)	11.4 (0.44)	–
	DF	83	84	–	83	84	83	83	84	–
	<i>F</i> -value	1.00	29.64	–	62.68	2.02	37.87	0.10	0.01	–
	<i>P</i> -value	0.321	<0.001	–	<0.001	0.159	<0.001	0.755	0.919	–
<b>Adult housing<sup>2</sup></b>										
<b>FC-L</b>		121.9 (16.33)	968.8 (7.63)	–	60.2 (1.21)	12.3 (0.45)	46.3 (1.26)	6.9 (0.81)	11.9 (0.42)	–
<b>FC-S</b>		79.7 (22.61)	954.3 (11.25)	–	62.6 (1.64)	11.3 (0.65)	49.8 (1.74)	4.8 (1.13)	10.7 (0.58)	–
<b>CC</b>		114.8 (16.48)	960.8 (7.55)	–	58.9 (1.22)	12.2 (0.45)	45.1 (1.27)	6.7 (0.82)	11.6 (0.42)	–
	DF	83	84	–	83	84	83	83	84	–
	<i>F</i> -value	1.46	0.65	–	1.98	1.02	2.87	1.51	1.72	–
	<i>P</i> -value	0.237	0.525	–	0.144	0.365	0.062	0.228	0.185	–
<b>Interaction R*A<sup>3</sup></b>										
<b>Conv * FC-L</b>		123.9 (22.40)	991.5 (11.23)	–	54.7 (1.63)	11.7 (0.64)	42.2 (1.73)	6.7 (1.12)	11.6 (0.57)	–
<b>Conv * FC-S</b>		101.8 (24.41)	990.3 (12.93)	–	55.1 (1.84)	11.2 (0.74)	43.0 (1.96)	5.6 (1.28)	11.1 (0.65)	–
<b>Conv * CC</b>		119.2 (19.29)	985.3 (9.41)	–	55.6 (1.41)	11.7 (0.55)	42.5 (1.49)	6.6 (0.96)	11.5 (0.50)	–
<b>Avi * FC-L</b>		119.9 (20.40)	946.0 (10.07)	–	65.6 (1.49)	12.9 (0.59)	50.5 (1.57)	7.2 (1.02)	12.3 (0.53)	–
<b>Avi * FC-S</b>		57.5 (35.19)	918.2 (18.25)	–	69.9 (2.53)	11.4 (1.04)	56.6 (2.71)	3.9 (1.78)	10.4 (0.90)	–
<b>Avi * CC</b>		110.4 (23.78)	936.4 (11.59)	–	62.3 (1.73)	12.7 (0.67)	47.8 (1.83)	6.7 (1.19)	11.6 (0.59)	–
	DF	83	84	–	83	84	83	83	84	–
	<i>F</i> -value	0.35	0.53	–	2.78	0.25	2.32	0.35	0.53	–
	<i>P</i> -value	0.896	0.593	–	0.068	0.777	0.104	0.703	0.588	–

<sup>1</sup>Pullets housed in a conventional rearing system (Conv) or an aviary rearing system (Avi) until 16 wk of age, Flocks 1 to 4.

<sup>2</sup>Adult hens placed into furnished cage- large (FC-L), furnished cage-small (FC-S), or conventional cages (CC) from 16 to 73 wk of age, Flocks 1 to 4.

<sup>3</sup>Interaction between rearing housing system and adult housing system.

tibiae of the Avi hens at the end-of-lay indicates that more mineral content was present but spread out over a greater area in the Avi hens.

The sequence of bone growth during development might also explain the greater bone mineral density values of the Conv hens. As the long bones undergo their final growth in diameter, increasing by more than 20% in the final wk before the onset of lay (Riddell, 1992), the growth outward is normally coupled with an incomplete bone framework of channels in the cortical ring that is subsequently filled in when outward growth ceases (Whitehead, 2004). It is possible that the lesser adult bone density values of the Avi hens were a result of greater outward growth in area, subsequently filled in with lower density medullary bone integrating into the pores in the cortical bone, resulting in a lower overall density detected by QCT in the regions of this “diluted” cortical bone. The accrual of medullary bone within the cortical space might have been exacerbated by the early lighting program and placement into adult housing at 16 wk in this experiment, which was carried out to encourage nest usage in furnished cages. It is possible that the bones of the Avi pullets had not entirely completed their structural growth at the time of the abrupt light stimulation, and in turn filled in the remaining channels with less dense medullary bone, thereby reducing the overall cortical density. Although a later initiation of the photo-stimulation for the Avi pullets might have allowed for more complete bone growth, this study was designed to minimize any management differences

between the aviary- and conventionally reared pullets. Since the aviary-reared pullets are slightly slower growing, as seen by the lower rearing BW of the aviary-reared pullets and the less ossified keel bones of the aviary-reared pullets (Casey-Trott et al., 2017b), a future study combining rearing treatments with a later onset of photo-stimulation may demonstrate further benefits to the bone composition of aviary-reared pullets. Even though cortical densities of all 3 bones were less in the Avi hens, increased bone width, which was confirmed in the Avi hens for cross-sectional area at 16 wk of age (Casey-Trott et al., 2017b) and in the adult hens reported here at the end-of-lay, has been shown to positively improve bone strength as wider bones are directly correlated with higher bending strength (Rauch, 2007).

Although overall body size can influence bone composition and strength as it adds to the loading strain placed on a bone (Cooper et al., 1995), it is not believed to be the driving factor in the difference in bone composition of hens within this study. It is important to emphasize that the larger bone cross-sectional areas of aviary-reared pullets were present at the end of rearing (Casey-Trott et al., 2017b) and maintained through the end-of-lay, even though the initial placement BW of the Avi hens were lower than those of the Conv hens. This suggests that even though the aviary-reared pullets from the population used within the current study had smaller BW at placement into adult housing compared to the conventionally reared pullets, the Avi pullet

**Table 3.** Bone traits of the tibia in 73-week-old laying hens subjected to different rearing and adult housing systems as measured by quantitative computed tomography.

		Density mg/cm <sup>3</sup> (±SE)			Cross-sectional area mm <sup>2</sup> (±SE)			Bone mineral content mg/mm (±SE)		
		Total	Cortical	Trabecular	Total	Cortical	Trabecular	Total	Cortical	Trabecular
Rearing <sup>1</sup>										
Conv		589.2 (7.68)	1063.2 (6.52)	208.19 (6.37)	39.1 (0.45)	17.3 (0.42)	21.2 (0.45)	23.1 (0.36)	18.4 (0.39)	4.4 (0.13)
Avi		590.6 (8.40)	1031.5 (7.03)	198.7 (6.93)	40.4 (0.47)	19.0 (0.45)	20.4 (0.50)	23.9 (0.39)	19.6 (0.43)	4.0 (0.15)
	DF	114	114	114	114	114	114	114	114	114
	F-value	0.01	12.90	1.08	5.61	9.12	1.29	2.36	4.86	4.33
	P-value	0.905	<0.001	0.301	0.019	0.003	0.259	0.127	0.029	0.039
Adult housing <sup>2</sup>										
FC-L		620.3 (8.65) <sup>a</sup>	1061.3 (7.19)	213.8 (7.11)	39.2 (0.48)	18.9 (0.46)	19.2 (0.51) <sup>a</sup>	24.3 (0.40) <sup>a</sup>	19.9 (0.44) <sup>a</sup>	4.0 (0.15)
FC-S		571.9 (12.42) <sup>b</sup>	1040.0 (10.00)	197.6 (10.10)	40.4 (0.64)	17.9 (0.65)	21.7 (0.73) <sup>b</sup>	23.2 (0.57) <sup>a,b</sup>	18.7 (0.61) <sup>a,b</sup>	4.3 (0.21)
CC		577.5 (7.92) <sup>b</sup>	1040.7 (6.72)	198.9 (6.57)	39.6 (0.46)	17.6 (0.43)	21.4 (0.47) <sup>b</sup>	22.9 (0.37) <sup>b</sup>	18.3 (0.41) <sup>b</sup>	4.2 (0.14)
	DF	114	114	114	114	114	114	114	114	114
	F-value	8.34	3.01	1.51	1.66	2.44	6.21	3.60	4.52	0.73
	P-value	<0.001	0.053	0.226	0.195	0.092	0.003	0.030	0.013	0.484
Interaction R*A <sup>3</sup>										
Conv * FC-L		604.1 (13.00)	1076.8 (10.40)	212.6 (10.54)	38.8 (0.66)	17.6 (0.67)	20.5 (0.77)	23.4 (0.60)	18.9 (0.64)	4.3 (0.22)
Conv * FC-S		574.6 (15.54)	1053.5 (12.33)	210.3 (12.56)	39.5 (0.77)	17.1 (0.80)	21.9 (0.92)	22.8 (0.72)	18.0 (0.76)	4.6 (0.27)
Conv * CC		589.0 (10.99)	1056.1 (8.95)	201.7 (8.97)	39.0 (0.58)	17.1 (0.58)	21.0 (0.65)	23.0 (0.51)	18.1 (0.55)	4.3 (0.19)
Avi * FC-L		636.5 (11.40)	1045.7 (9.22)	214.9 (9.28)	39.5 (0.59)	20.1 (0.60)	17.9 (0.67)	25.2 (0.52)	20.9 (0.57)	3.8 (0.20)
Avi * FC-S		569.2 (19.38)	1023.5 (15.29)	185.0 (15.64)	41.4 (0.95)	18.8 (0.99)	21.5 (1.14)	23.6 (0.90)	19.3 (0.94)	3.9 (0.33)
Avi * CC		566.0 (11.40)	1025.2 (9.25)	196.2 (9.30)	40.2 (0.60)	18.0 (0.60)	21.7 (0.67)	22.8 (0.53)	18.5 (0.57)	4.2 (0.20)
	DF	114	114	114	114	114	114	114	114	114
	F-value	2.84	0.00	0.65	0.36	0.93	2.57	1.89	1.06	0.70
	P-value	0.062	0.995	0.524	0.699	0.397	0.081	0.155	0.351	0.497

<sup>a,b</sup>Within a column, for each treatment variable (Adult housing, Interaction R\*A), means lacking a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Pullets housed in a conventional rearing system (Conv) or an aviary rearing system (Avi) until 16 wk of age, Flocks 1 to 4.

<sup>2</sup>Adult hens placed into furnished cage- large (FC-L), furnished cage-small (FC-S), or conventional cages (CC) from 16 to 73 wk of age, Flocks 1 to 4.

<sup>3</sup>Interaction between rearing housing system and adult housing system.

bones were already larger than those of the Conv pullets based on the cross-sectional area values reported (Casey-Trott et al., 2017b). Because structural bone growth ceases at the onset of lay (Whitehead, 2004), it is unlikely that the adult housing system or the larger overall BW of the Avi hens as adults influenced the cross-sectional area values reported at 73 weeks. Instead, it is possible that the Avi pullets were set on a path to developing a larger skeletal frame overall, as reflected by the larger final BW of the Avi hens compared to the Conv hens, potentially offering the opportunity to provide a larger skeletal frame to store and mobilize calcium during the laying period.

### Effect of Adult Housing System on Bone Characteristics

Opportunities for exercise allowed by adult housing systems have been more extensively studied, and researchers frequently cite the positive effects of exercise on bone characteristics. Allocation of perches in conventional cages has been shown to increase bone mineralization (Hester et al., 2013), and housing in furnished cages (Jendral et al., 2008), aviaries (Leyendecker et al., 2005), or free-range systems (Shipov et al., 2010) during the laying phase allows for enough exercise to improve bone mineral density, cortical area, and breaking strength compared to hens housed in conventional cages. Although several effects of adult housing system on bone characteristics at the end-of-lay are reported

here, the number and magnitude of effects are less than the results reported for the effects of rearing system on pullets (Casey-Trott et al., 2017b) and adult bone composition, highlighting the importance of providing opportunities for exercise during development vs. adulthood. In humans, bone growth during adolescence primarily alters the bone geometry by adding to the periosteal and endocortical bone layers, thereby increasing bone width, whereas bone formation in adults is driven by internal trabecular remodeling (Rauch, 2007). The same is true for laying hens, with structural growth occurring only prior to sexual maturity followed by accrual of medullary bone filling in the trabecular cavity (Whitehead, 2004). The presence of rearing effects in both pullet and end-of-lay hens on measurements of total and cortical bone cross-sectional area and the lack of adult housing system effects on the total or cortical cross-sectional area dimensions fit this described sequence of bone growth and appear to confirm previous results that loading exercise prior to sexual maturity primarily alters the shape and size of the bone rather than substantially affecting mineral components (Regmi et al., 2015; 2016).

However, as previously stated, adult housing system did have some effects on bone composition in adult hens. The radius and tibia of hens in FC-L were positively affected by the housing system presumably due to the increased allowance of exercise due to the larger floor area provided. For every significant or nearly significant result for bone mineral density, cross-sectional



**Table 4.** Comparison of bone breaking strength in adult laying hens at 73 wk of age.

		Maximum bone breaking strength (kg)		
		Humerus ( $\pm$ SE)	Radius ( $\pm$ SE)	Tibia ( $\pm$ SE)
Rearing <sup>1</sup>				
Conv		9.2 (0.45)	5.1 (0.13)	14.1 (0.75)
Avi		6.3 (0.44)	5.1 (0.12)	14.4 (0.75)
	DF	40	57	57
	F-Value	34.6	0.05	0.59
	P-Value	<0.001	0.816	0.445
Adult housing <sup>2</sup>				
FC-L		7.7 (0.48)	5.2 (0.14)	14.8 (0.76) <sup>a</sup>
FC-S		7.4 (0.55)	5.2 (0.17)	14.4 (0.78) <sup>a</sup>
CC		8.1 (0.49)	5.1 (0.18)	13.5 (0.77) <sup>b</sup>
	DF	40	57	57
	F-value	0.56	0.11	5.59
	P-value	0.566	0.893	0.006
Interaction R*A <sup>3</sup>				
Conv * FC-L		9.2 (0.62)	5.1 (0.18)	14.4 (0.82)
Conv * FC-S		9.6 (0.74)	5.1 (0.19)	14.6 (0.85)
Conv * CC		8.7 (0.62)	5.1 (0.17)	13.3 (0.81)
Avi * FC-L		6.3 (0.62)	5.2 (0.17)	15.2 (0.81)
Avi * FC-S		5.3 (0.69)	5.2 (0.18)	14.2 (0.83)
Avi * CC		7.5 (0.65)	5.1 (0.18)	13.7 (0.82)
	DF	40	57	57
	F-value	3.1	0.07	0.99
	P-value	0.058	0.929	0.378

<sup>a,b</sup>Within a column, for each treatment variable (Adult housing, Interaction R\*A), means lacking a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Pullets housed in a conventional rearing system (Conv) or an aviary rearing system (Avi) until 16 wk of age, Flocks 1 to 4.

<sup>2</sup>Adult hens placed into furnished cage-large (FC-L), furnished cage-small (FC-S), or conventional cages (CC) from 16 to 73 wk of age, Flock 1 to 4.

<sup>3</sup>Interaction between rearing housing system and adult housing system.

area, and bone mineral content of the radius and tibia, the FC-L had the greatest values overall compared to FC-S and CC, except for trabecular cross-sectional area and trabecular bone mineral content. This suggests that the increased strain from loading exercise as adults continues to increase total and cortical bone mineral density by enhancing osteogenesis or preserving the beneficial effects of rearing exercise on the total and cortical bone cross-sectional area as the hens age. Jendral et al. (2008) demonstrated an increase in bone density and area with minor modifications to cages, such as increasing space and adding perches and nests to conventional cages or providing cages with or without an elevated dust bath. Similar results were found here with higher bone mineral density of the radius and tibia for hens in FC-L as compared to hens in FC-S. This is the first study, to the best of our knowledge, to provide evidence that access to increased total area within a cage, even when furnishings and space allowance are held constant, encourages enough increased exercise and use of space to increase bone mineral density in adult laying hens. Since the consistent opportunity for walking is considered a sufficient method of creating loading-strain on bones stimulating bone growth (Shipov et al., 2010), it is likely that the additional area for locomotion provided within the FC-L stimulated additional deposition of bone mineral content. The greater bone mineral content in the radius of hens housed in FC-L also suggests that this additional area within the FC-L allowed for a greater amount of wing-loading exercises,

perhaps in the form of wing flapping, which is supported by research reporting that furnished cages holding >60 hens at a space allowance of 599 cm<sup>2</sup>/hen provided sufficient space to support wing flapping (Mench and Blatchford, 2014). The overall greater values for the FC-L compared to the CC reported here highlights the importance of the provision of furnishings and space allowance to stimulate bone composition; however, the additional finding that FC-L produces even greater values than the FC-S indicates the importance of providing a larger cage area to encourage better use of space and locomotion within a cage to further stimulate bone mineral deposition.

Trabecular area had the opposite effect, with FC-S or CC possessing the highest trabecular values and FC-L possessing the lowest. As an example, the radius of hens in FC-S and CC had lower trabecular densities yet higher trabecular areas ( $P = 0.053$ ) when compared to the radius of hens in FC-L. This pattern is supported by previous research (Jendral et al., 2008) and potentially highlights a difference in calcium metabolism triggered by exercise levels, as it has been suggested that larger trabecular areas might be indicative of inadequate prevention of bone resorption on the endosteal surface of the cortical bone (Jendral et al., 2008). Given that the trabecular area of the radius was smaller for hens in FC-L ( $P = 0.053$ ), the greater trabecular density is likely due to similar content being placed into a smaller area as compared to the radius of hens of CC and FC-S.

With all the evidence of positive bone quality characteristics provided by the QCT analyses at both the pullet and end-of-lay stages, it brings up the question of why consistent differences between rearing systems were found at 16 wk in the analysis of breaking strength (Casey-Trott et al., 2017b), but not between rearing system or between adult housing system at the end-of-lay. In addition, although the tibiae breaking strength results are in line with the highest QCT bone values reported for the FC-L, it is unclear why the humeral breaking strength is higher in the Conv hens at 73 wk of age. It is possible that the bone mineral content was more evenly distributed in the pullet bones, whereas the adult bones might have produced more varied breaking strength results due to inconsistent bone mineral distribution or areas of structural weakness. Or perhaps the breaking strength technique used here was not ideal for assessing the resiliency of adult bone. Techniques described by Crenshaw et al. (1981) and Newman and Leeson (1998) were used in the current study to minimize any variation from inadvertent drying of wet bone during testing; however, drying the bones might not have been appropriate for a robust understanding of breaking strength in the adult bones. Several studies of bone breaking strength in adult laying hens use a “wet” technique to assess the bending strength (Knott et al., 1995; Fleming et al., 1998a,b; Silversides et al., 2006, 2012; Riczu et al., 2008; Jendral et al., 2008; Habig and Distl, 2013), instead of the drying method that was used here. Although bone geometry and content are critical components to the strength of a bone, collagen and the collagenous matrix of cross-linkages also play an important role in the resistance to bending and compression stresses (Knott et al., 1995), and the gene expression of collagen type I is known to increase in response to loading in chick tibiae (Zaman et al., 1992). More complex techniques for the assessment of breaking and bending strength that include the consideration of collagen and complete bone components would be favorable in future research. Techniques described by Regmi et al. (2015) can provide much more detailed information. Although using a wet method, with more comprehensive testing is recommended for future studies assessing breaking strength, the consistency of techniques used for all bones handled here still allows for a valid comparison of the bones within the study.

Overall, the greater total and cortical bone cross-sectional area observed in the Avi pullets at 16 wk of age (Casey-Trott et al., 2017b), and the fact that the majority of effects of aviary-rearing were maintained through to the end-of-lay indicates that opportunities for diverse, loading exercise during the rearing phase substantially alters the geometry of growing pullet bones. This increase in size of the aviary-reared pullet skeleton potentially affords greater space for bone mineralization and medullary bone deposition in adult hens. Perhaps other avenues of research targeting methods to increase the rate of calcium ab-

sorption or medullary bone deposition can be used in conjunction with this increased skeletal growth to capitalize on the newly available skeletal framework.

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